HAND INFECTION APPARENTLY DUE TO BACILLUS FUSIFORMIS.*†

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While the actual rôle of *Bacillus fusiformis* in the production of lesions in the human body is still *sub judice*, we cannot fail to recognize its importance as a factor, if we glance at the increased number of pathologic lesions from which it has been isolated. The organism has been observed in ulcero-membranous angina, hospital gangrene, noma, appendicitis, diphtheria, foetid bronchitis, gangrenous laryngitis, pyorrhea alveolaris, brain abscess, and in the healthy mouth.

According to Jungano and Distaso,[1] Plaut first described it in 1894 in a case of ulcerous angina, while Veillon and Zuber were probably the first to isolate the bacillus in pure culture. Vincent's descriptions appeared about two years after Plaut's. The organism has also been grown in pure culture by Ellerman, Weaver,[2] Tunnicliff,[3] Lewcowitz, Leiner, Repaci, and Ghon and Mucha.

Five cases of unusual infection with fusiform bacilli and spirochaetes have been studied by me.

Case 1.—S. F., male, age 4, was admitted to the Cincinnati Hospital October 5, 1909. He had a typical lobar pneumonia with delayed resolution, which was followed by an abscess of the lung. Death ensued sixteen days after admission. Smear preparations of the pus obtained before death from the thorax, by aspiration, revealed fusiform bacilli and spirochaetes in great numbers and streptococci. The fusiform bacilli measured from 2.7 μ to 7.0 μ by 0.5 μ. Apparently two varieties of spirochaetes were observed; thick ones with irregular loose windings corresponding to the refringens type, and others composed of two or three turns and of regular amplitude. The same organisms were found in the sputum, together with a third type of spirochaete, viz., dentium.

Case 2.—P. S., a white male, age 45, was admitted to the Cincinnati Hospital October 3, 1910, with marked dyspnoea, laryngeal stridor, and an irregular pulse.

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† Photomicrographs by Dr. Chas. Goosmann, Cincinnati, Ohio.

[1] Jungano and Distaso, *Les anaérobies*, 1910, p. 155.

[2] Weaver, *Jour. Am. Med. Assn.*, 1906, 46, p. 481.

[3] Tunnicliff, *Jour. Infect. Dis.*, 1906, 3, p. 148.

He died one hour after admission. Post-mortem examination revealed a syphilitic ulceration and edema of the larynx and trachea, and other tertiary lesions. Coverslip preparations were made from the ulcer at the base of the larynx, demonstrating fusiform bacilli resembling those described by Vincent, and spirochaetes of the refringens type.

Case 3.—F. S., the patient, had a chronic foetid bronchitis. Smear preparations of the purulent secretions showed the presence of *Bacillus fusiformis* in great numbers and an absence of spirochaetes.

These cases are mentioned briefly with a twofold purpose, viz., demonstration of the organism in question and its relative significance when associated with respiratory disorders.

Case 4.—J. K., age 38, laborer. On March 17, 1911, at Gadson, Ala., he struck a man in the teeth, injuring the index and middle fingers of the right hand. Intense swelling, edema, and a foul discharge characterized the condition. Smear preparations made from the discharge on April 9 showed the presence of fusiform bacilli and streptococci. No spirochaetes were demonstrable.

Before proceeding with the case that was studied culturally as well as microscopically, I should like to refer to the case reported by Hultgens,[1] a seven-year-old girl who showed partial gangrene of the left index finger. In smear preparations he found fusiform bacilli and spirochaetes. He does not describe the spirochaetes but calls them *Spirochaeta denticola.* His patient had carious teeth and had been in the habit of biting her finger nails. Film preparations made from her carious teeth showed the presence of these same organisms. Apparently no cultural studies were attempted. The source of infection makes this and the following case interesting especially in view of the fact that no cases are reported of direct transmission from one individual to another.

Case 5.—A. W., age 34, a bar tender by occupation. On September 6, 1910, he struck a man in the teeth, injuring the base of the little finger of his left hand. Two days later the finger was swollen and discharging a foul pus. He was admitted to the Cincinnati Hospital, with a temperature of 99.8°, pulse and respiration normal. Two free incisions were made, and the hand immersed in a continuous bichloride bath. Nine days after injury the wound was not doing well; had a chronic persistent appearance and was still discharging. Eighteen days following injury the left hand and forearm were swollen and markedly edematous, and the discoloration assumed a purplish hue. The wounds were ragged, irregular, and surrounded by cauliflower-like excrescences, with evidence of deep destruction of the tissues. The appearance of these wounds was suggestive of epithelioma. The patient was unwilling to submit to further surgical interference and was discharged. He made a slow recovery, as was learned later, and was well 54 days after the injury.

[1] Hultgens, *Jour Am. Med. Assn.*, 1910, 55, p. 857.

Smear preparations from the wound, made September 20, showed numerous leukocytes and almost pure culture of fusiform bacilli and spirochaetes. The bacilli, for the most part, are long and regular, with pointed ends, and thicker in the middle. They lie side by side, between the cells, or end to end, and sometimes in irregular clumps. In size the bacilli vary from 2.7 μ to 8.1 μ in length by 0.6 μ in breadth. The spirochaetes are very numerous, and at least two types are visible. Most of them show three or four turns which are of irregular amplitude, corresponding to the refringens type. Their extremities are parallel to the long axis of the spirochaete with two or three thick regular turns corresponding to *Spirochaeta recta*, and another, with four or five regular turns, but which are much thinner, corresponds to *Spirochaeta tenuis*.[1] The spirochaetes measure from 9.0 μ to 16.2 μ in length by less than 0.4 μ

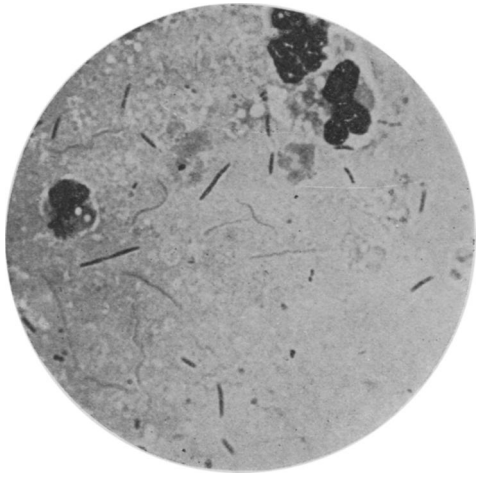

FIG. 1.—Film preparation from infected hand, Case 5, demonstrating *B. fusiformis*, ×1,000. Stained with carbol fuchsin.

FIG. 2.—Smear from infected hand, Case 5, demonstrating spirochaetes, ×1,000. Stained with polychrome methylene blue for 24 hours.

in breadth (Spencer apochromatic 2mm. Ocular No. 8). Smear preparations 46 days after the injury showed the same organisms. A mixed infection was evidenced by the presence of a limited number of cocci and small bacilli. The spirochaetes were not so abundant, appeared thinner than when first observed, and did not stain so well.

Slants of Dorset's egg medium were inoculated with the purulent secretion taken from this case of hand infection. After anaerobic incubation at 37° C. for three days, colonies of two kinds appeared, cocci and fusiform bacilli. By transplanting from the small colonies of spindle-shaped bacilli pure cultures were obtained.

Morphology and staining reactions.—In smear preparations from 24-hour cultures, the fusiform bacilli are delicate pointed rods and usually straight. As they mature, they become long, slender

[1] Gerber, *Centralbl. für Bakt.*, 1 Abt., Orig., 1910, 56, p. 508.

rods with pointed ends, and somewhat thicker in the middle. Very frequently they are slightly curved. They measure 4.5 μ to 46.8 μ in length by 0.6 μ in breadth.

In some of the cultures wavy forms may be observed. The morphologic characteristics of these are not unlike those of the bacilli. The protoplasm reacts to aniline dyes and to light in the same way; and the presence of metachromatic granules are suggestive of fusiformis. The longer bacilli most frequently contain

FIG. 3.—Pure culture of *B. fusiformis*, ×1,000. Stained with polychrome methylene blue for 48 hours.

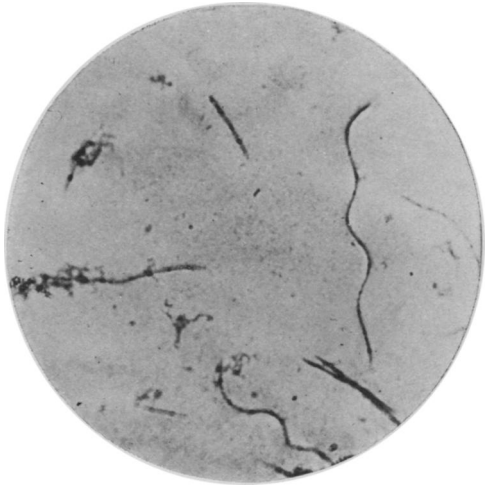

FIG. 4.—Pure culture of *B. fusiformis*, ×1,000. Stained with polychrome methylene blue for 48 hours, showing wavy forms.

four or more metachromatic granules while the shorter forms contain two. In old cultures the granules are stained less intensely or may be entirely absent. Suspended in Gram's iodine solution they do not give the starch reaction.

In making film preparations from solid cultures the bacilli often remain adherent throughout their length, forming bundles. Occasionally two bacilli may be seen lying side by side, so close together as to make one think that they divide longitudinally; however there are no indications of terminal splitting. Some appear only half as thick as others. On the other hand, there is evidence in the film preparations that transverse division occurs.

Some of the bacilli seem to be constricted, and here the protoplasm is thinner and less granular. The fusiform bacilli as well as the spirochaetes are stained by Loeffler's methylene blue, polychrome methylene blue, carbol gentian violet, carbol fuchsin, and by the Giemsa and Romanowski stains. Carbol fuchsin and polychrome methylene blue are the most satisfactory. Specimens were stained in polychrome methylene blue for 24 hours, washed with water, and mounted in balsam. The spirochaetes stain less intensely than the bacilli.

The definition of *B. fusiformis* is beautifully demonstrated by the aniline black method. Neither the bacilli nor the spirochaetes retained the stain in Gram's method, contrary to the statement of Jungano and Distaso.

Cultural properties.—The cultures were grown anaerobically by the pyrogallic acid method. The colonies, not unlike streptococcus colonies, are small and delicate with slightly raised centers about one or two mm. in diameter. The best growth was obtained at 37° C. In the hanging drop the organisms show no active or progressive motility but considerable vibratory motion especially at one end.

So far as the viability of *Bacillus fusiformis* on artificial media is concerned, Tunnicliff found them alive 55 days after inoculation. Cultures on Dorset's egg medium and Loeffler's blood serum have been found by myself, viable 20, 40, and 47 days after transplanting. The viability of the culture is conserved for some time in the refrigerator or maintained by frequent transfers. Twenty subcultures made during the past seven months have been grown successfully.

Loeffler's blood serum and Dorset's egg medium are productive of the most luxuriant growth, the colonies appearing as delicate irregular white masses with slightly raised centers. Growth is scarcely visible at the end of 24 hours, but after 48 or 72 hours' incubation the colonies measure 1 or 2 mm. in diameter. A flocculent growth is usually observed in the water of condensation. Ascites broth offers a very favorable means of cultivation. The growth is heavy, luxuriant, flocculent, and sinks to the bottom. By agitating the tubes this may be divided into small particles.

On rabbit's blood agar luxuriant growth was obtained resembling that seen on Loeffler's blood serum. When such a culture was placed under aerobic conditions the culture medium darkened and was black a week later. In Dunham's peptone solution there was a slight flocculent growth which settled on the bottom. In litmus milk limited growth occured after 72 hours at 37° but no coagulation took place. When broth with a reaction of one per cent acid to phenolphthalein and containing one per cent of dextrose, lactose, saccharose, maltose or mannite was inoculated, no growth occurred, but the addition of 0.5 c.c. of defibrinated rabbit's blood to six c.c. of these various sugar broths yielded luxuriant growths. Acid production was marked at the end of 72 hours excepting in saccharose. The litmus in dextrose and lactose broths was entirely reduced. The same luxuriant growth was obtained in litmus milk when 0.5 c.c. of defibrinated rabbit's blood was added. No growth appeared upon +1. agar, or upon one per cent glucose agar.

When the stoppers were removed from the culture tubes a foul odor was given off suggestive of skatol. The reaction for indol with potassium nitrate and sulphuric acid was negative.

Resistance.—As far as I know the resistance of the fusiform bacilli to moist heat has not been determined. Attempts were made to settle this point by heating ascites broth cultures and suspensions in 0.85 per cent sodium chloride solution. As the controls in this series often showed no growth, the following technic was adopted.

Seventy-two-hour cultures on Loeffler's blood serum were used. Anaerobic conditions were suspended and the rubber stopper and pyrogallic acid plug replaced by sterile cotton and a rubber cap. The tubes were then suspended in a water bath for 15 minutes at 50°, 55°, 60°, and 65° C. respectively. Subcultures were made from each tube and the result obtained was confirmed by an additional subculture from the first subculture. Controls were used throughout.

The bacilli are killed by exposure to moist heat for 15 minutes at 55° C. They are not affected by an exposure at 50° C. for the same length of time.

The fusiform bacillus is able to withstand the action of anti-

formin, one per cent solution, for five minutes without altering its viability. Seventy-two-hour cultures on Loeffler's blood serum were covered with the germicide for two and five minutes respectively, after which they were washed with sterile distilled water. The transplants showed luxuriant growths in 48 hours. When the colonies were covered with hydrogen peroxide, 15 per cent solution, for five minutes and washed with sterile water, no growth appeared in the subcultures. A very luxuriant growth was obtained in the transplants from colonies which had been exposed to hydrogen peroxide for one minute. Cultures were subjected to the action of hydrogen peroxide for one, five, ten, fifteen, and twenty minutes on two different occasions to verify the above results.

Inoculation experiments.—Two full-grown guinea-pigs were inoculated with ascites broth cultures. The first pig received one c.c. of a 72-hour culture intraperitoneally, and the other one c.c. subcutaneously. A white rat and a wild rat, *M. norwegicus*, were given one c.c. intraperitoneally. A large rabbit weighing 1560 gms. was inoculated with 0.75 c.c. intravenously. There was an entire absence of any local symptoms, and at the autopsy, 30 days after inoculation, no pathological conditions were visible in these animals. The fusiform bacillus was recovered from the peritoneal smear of the first guinea-pig, but there was no evidence of multiplication. Tunnicliff's results were negative in guinea-pig experiments.

In the review by Jungano and Distaso they conclude that *Bacillus fusiformis* is pathogenic for the guinea-pig and the mouse. The strain of Leiner was very virulent for the lower animals and one of Repaci's cultures was also pathogenic. Veillon and Zuber were only able to produce a very mild grade of infection with their strains.

At the present time there seems to be a difference of opinion as to the relation of the spirochaetes to *Bacillus fusiformis*. Some authors believe that they are different organisms entirely, and that the presence of the spirochaetes increases the virulence of the bacilli. Others maintain that they are two forms of one organism in its cycle of evolution. Tunnicliff claims to have observed spirilla develop from the fusiform bacilli in her cultures

after they had grown from two to five days. I was unable to note spirochaetes in any of the cultures. The terms spirilla and spirochaeta have been used indiscriminately in the literature, but I think we should adhere rigidly to the word spirochaeta because the symbiosis occurring in the mouth is one of fusiform bacilli and spirochaetes. As was mentioned above, some of the cultures contained wavy forms, but no true spirochaetes were demonstrable. Ellerman holds to the same opinion.

Future study may show that certain metabolic products of *B. fusiformis* favor the growth of the spirochaetes. Repaci isolated spirilla (spirochaetes?) from the mouth, which he claimed to be separate distinct organisms from *B. fusiformis* by reason of their chemical and biological peculiarities. Furthermore the successful cultivation of *Sp. dentium*, *Sp. refringens*, and *T. pallidum* certainly seems to separate this group of organisms from any developmental forms of the bacilli.

SUMMARY.

1. The substance of this article consists, essentially, in a preliminary study, microscopic and bacteriologic, of *Bacillus fusiformis*. Its biochemical properties with special reference to its action upon proteids will be discussed later.

2. Two cases are recorded of direct transmission from one individual to another.

3. In two cases the fusiform bacilli were not accompanied by spirochaetes.

4. The organism grows luxuriantly upon Dorset's egg medium, and in various sugar broths containing a small amount of defibrinated rabbit blood.

5. Mention is made of its resistance to moist heat, antiformin, and hydrogen peroxide.

In conclusion I wish to express my gratitude to Dr. Wm. B. Wherry for his many helpful suggestions.